Claim Amendments

The listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently amended) A process for the production of egg yolk antibodies binding to small molecule organo chlorine pesticides, the said process comprising the steps of:
(a) selecting a suitable poultry bird;
(b) immunizing the poultry bird with known complete adjuvant, each ml of said adjuvant comprising heat killed and dried 1 mg of Mycobacterium tuberculosis (H37Ra, ATCC 25177), 0.85 ml paraffin and 0.15 ml mannide monooleate;
(c) immunizing the bird with 1000 μg of the hapten protein conjugate 2,4,5 trichlorophenoxyacetic ~~trichlorophoxyacetic~~ acid β-alanine administered to breast muscle;
(d) immunizing the bird again with the hapten-protein conjugate as given in step (c) with 500 μg of the hapten conjugate;
(e) immunizing the bird with the hapten-protein conjugate at the intervals of two, three and five weeks;
(f) immunizing the bird thereafter with the hapten-protein conjugate at five weeks intervals as long as the bird lays eggs; and
(g) harvesting antibodies from the egg yolk of the bird.

2. (Previously presented) A process as claimed in claim 1, wherein the hapten protein conjugate has binding properties to hexachlorohexane.

3 -4. (Canceled)

5. (Previously presented) A process as claimed in claim 1, wherein the production of conjugate hapten 2,4,5-trichloro phenoxy acetic acid β-alanine (TCB) hapten binding to hexachloro hexane, as per step(c), is as follows:

(a) adding ~~of~~ β-alanine spacer arm to 2,4,5-trichlorophenoxyacetic ~~ic~~ acid by suspending 10 mM, 2.55 g of 2,4,5-trichlorophenoxyacetic ~~e~~ acid in 5.95 ml thionyl chloride;

(b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation;

(c) stirring the product with β-alanine in 1M NaOH at 0 ° C;

(d) warming the product for over 16 hours at room temperature;

(e) isolating the resulting acid by acidification;

(f) partitioning into ethyl acetate;

(g) washing with water and brine;

(h) giving an yield of crude product hapten containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as impurity;

(i) dissolving the impurity in acetone to obtain colorless flakes of the trichlorobenzene (TCB) hapten;

(j) filtering and washing the colourless TCB hapten with acetone and drying in air;

(k) using silica gel precoated aluminum plates and a mixture of chloroform and methanol in a ratio of 85:15 as eluent showed a single spot in TLC analysis Rf-0.45 detected by spraying with 2% o-tolidine in acetone and exposure to UV light or sunlight at a melting range of 169-70 ° C;

(l) synthesizing the active ester of hapten 2,4,5-T-β-alanine at melting range of 102-104 ° C by dissolving in dichloromethane;

(m) adding N- and the mixture is cooled in an ice-bath;

(n) adding dimethylsulphoxide hydroxysuccinimide (DMSO) dropwise to the mixture until the hapten is dissolved;

(o) adding dicyclohexylcarbodiimide to the mixture followed by adding dimethylaminopyridine catalyst;

(p) stirring the mixture overnight and allowing the temperature to slowly raise to room temperature;

(q) filtering and evaporating the acetone; and

(r) separating the active ester as a colorless solid.

6. (Currently amended) A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, is as follows:

(a) obtaining egg yolk without rupturing the yolk;

(b) adding 100 ml of Tris buffer saline for every 10 ml of yolk;

(c) removing the precipitate by centrifugation;

(d) adding to the supernatant the precipitating solution of magnesium chloride and phosphotungstic acid for centrifuging;

(e) discarding the pellet;

(f) adding to the supernatant a water soluble protein fraction 12% polyethylene glycol;

(g) incubating for 10 minutes and then centrifuging again;

(h) precipitating out the antibody;

(i) adding 10 ml of 10 mM phosphate buffer to dissolve the precipitate;

(j) cooling the antibody solution 0 °; [[Cl;]]

(k) adding 10 ml of pre-cooled ethanol;

(l) centrifuging the solution at 4 ° C and dissolving the sediment in 10 mM phosphate buffer; and

(m) dialyzing against phosphate buffer for 24 h at 4 ° C. to obtain the yield of antibodies.

7. (Previously presented) A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, can also be conducted as follows:

(a) obtaining the egg yolk from the eggshell without rupturing the yolk membrane;

(b) adding for every 10 ml of yolk, 10 ml of distilled water;

(c) adding about 0.15% of kappa-carrageenan and left to stir for 30 minutes at room temperature;

(d) filtering and centrifuging the solution at for 15 minutes;

(e) passing through the DEAE-sephacel column prepared with 20 mM phosphate buffer pH 8.0;

(f) eluting with 0.2 M phosphate buffer pH 8.0;

(g) collecting the eluate and the absorbance read at 280 nm; and

(h) pooling and storing the peak fractions containing the antibody at 4 ° C.

8. (Previously presented) A process as claimed in claim 6, wherein the lipid from the egg yolk is precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride and centrifuged and an antibody yield of up to 75% is obtained from the supernatant.

9. (Previously presented) A process as claimed in claim 6, wherein the pH of the water soluble protein fraction obtained after the removal of the lipids is adjusted to pH 5.0 to further precipitate out the antibodies for obtaining a yield of 80-90%.

10. (Previously presented) A process as claimed in claim 7, wherein the yield of antibody is up to 73%.

11. (Previously presented) A process as claimed in claim 1, wherein the hyper immune eggs are collected daily and stored 4 ^{o} C until further use.

12. (Previously presented) A process as claimed in claim 1, wherein commencing the production of the antibody from the seventh day after the immunization and continued for 60 days.

13. (Original) A process as claimed in claim 1, wherein the titer of the antibody produced is 165-225 mg/ml.

14. (Previously presented) A process as claimed in claim 1, wherein production of the egg yolk antibody is more or equally sensitive to the polyclonal or monoclonal antibodies produced using mammals.

15. (Previously presented) A process as claimed in claim 1, wherein the egg yolk antibodies produced bind to small molecule organic pesticides.